Structure and dynamics of active actin-myosin networks

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Summary

There are infinite numbers of natural *matrioskas* in our Universe. Inside our cosmos there are planets. Our planet happens to be the home to numerous species of living organisms. And the latter are made of building blocks named cells. Cells are the actual fundamental units that store the genetic information which defines an organism's body plan. A larger amount of genetic information paves way for more complexity and contrary to simple unicellular bodies like bacteria, cells of higher order organisms can cooperate with each other to organize into structures as complex as thousands of years old forests of aspen, a whale or a human brain.

In multicellular organisms, cells act as small autonomous, but not independent, machines. By dividing and moving, especially during an organism's early development, cells organize together to form the adequate body form. This is only possible because, far from being a little "bag of water", a cell contains components that both maintain shape and allow for dynamic changes in shape. The material that is primarily responsible for such plasticity is the actin cytoskeleton. The actin cytoskeleton is a three dimensional "fishnet-like" structure of filamentous proteins which provides the cell with mechanical integrity. Actin filaments assemble by addition of monomeric units at both ends of the polymer. However, as one end grows at a faster rate, it is named the plus end. These filamentous proteins can organize into different higher order structures (review Fig. 5.13). So-called filopodia, fine tubular membrane extensions filled with actin filaments, allow cells to probe the surrounding environment. Lamellipodia are sheet-like protrusions at the front of motile cells which propel cells forward. In addition to these actin assemblies, dividing cells form rings of actin at their middle which serve to constrict the cell and help pinch off two daughter cells. Such rings are contractile due to the presence of myosin II. This molecular motor protein is able to transform chemical energy (ATP) into mechanical force which remodels the organization of actin filaments. Myosin II molecules have a tail domain and two head or motor domains. These domains function as hands and arms

pulling on a rope as they walk toward the plus end of actin filaments. Under physiological conditions, myosin tails attach to each other and form bipolar filament structures. The head domains are at the two ends and attach to actin filaments. As ATP, the energy source, binds to these head domains, they change conformation to pull on the actin filaments. The movement resembles the arms and hands of a person pulling on a rope to dock a boat. Instead of a single pair of hands/arms, myosin bipolar filaments have up to hundreds of myosin head domains pointing in opposite directions which allows them to tug on the surrounding actin network and rearrange its architecture. In fact, many processes regulating cellular shape are driven by myosin II-dependent rearrangements of the actin cortex. These include the formation of stress fibers or the contractile cortex at the rear ends of migrating cells. However, it is unclear how motor-generated forces reorganize networks of actin filaments to generate functional contractile structures.

The aim of my PhD research was to understand how physical mechanisms on the molecular scale lead to structural changes in the actin network and translate into active behavior at the cellular scale. To investigate the structure of active networks, I performed confocal microscopy of minimal *in vitro* networks of purified fluorescently labeled actin and myosin proteins. I characterized the dynamics of these networks using microrheology approaches, and elucidated the effect of membrane confinement on the structure of the actin cortex by polymerizing actin inside cell-sized hardwall microchambers. *Chapter 2* provides a summary of protein characterization techniques and of the *in vitro* approaches used.

In *chapter 3*, to elucidate the mechanisms which govern the formation of contractile actin structures, I set up a simplified model of the actin cortex that is devoid of biochemical regulation. This minimal model is composed of fluorescently labeled skeletal muscle myosin and actin proteins. I was able to show with time-resolved confocal microscopy that contractile activity of the motors resulted in actin network coarsening through a process of condensation-like steps. First, myosin motors form dense foci by moving along the actin network and permanently coalescing. Once myosin clusters are immobilized in the network, they accumulate actin filaments in a shell around them. Eventually, these intermediate actin-myosin clusters coalesce as a consequence of motors pulling on surrounding actin filaments that are not fully straightened and still thermally fluctuate.

We propose that the physical origin of this multistage cluster formation is the highly asymmetric response of actin filaments to force. While actin filaments can support large tensions when they are stretched, they easily buckle under minute compressive forces. In networks where actin filaments are weakly attached to each other, the connected network buckles under the tugging forces of the myosin motor-generated forces which are much larger than the buckling threshold. The same buckling effect of actin leads to the coalescence of isolated actin-myosin clusters into larger superaggregates. The resulting structures bear a striking resemblance to the structure and dynamics of myosin clusters observed in various types of living cells. Such in vivo foci are involved in myosin-dependent morphogenetic processes and in cell division. Our results suggest that this characteristic organization of actomyosin networks is an intrinsic outcome of the forces exerted by myosin motors on actin filaments.

Learning about the mechanism underlying motor-driven actin reorganization made it possible to quantitatively investigate the spatiotemporal characteristics of actomyosin network dynamics. In *chapter 4*, I thus focused on the microrheological properties of active networks by imaging and video tracking of embedded inert microspherical probes. This study provided insight into the out-of-equilibrium network fluctuations caused by myosin contractile activity, which is superposed on the omnipresent thermal jittering common to all natural systems.

I observed non-thermal microsphere trajectories, with periods of non-directed (random) motion interspersed with episodes of active, directed motion. These clear signatures of the contractile activity of the motors in the form of bouts of sudden, directed motion of the probes were usually followed by relaxation. These motions reflect the biochemical cycle of the myosin filaments. In contrast to assumptions of network homogeneity made in existing active gel theories, the motor-driven forces propagate in a highly heterogeneous filamentous network. These physical properties may contribute to the non-continuous, pulsatile cell shape changes that have been observed during morphogenetic events in developing embryos.

We developed new automated analysis to segment microsphere particle trajectories into active and non-active portions of the movement. This analysis allowed us to identify a pronounced age-dependence of activity. During the first 30 minutes after sample preparation, I observed fre-

quent periods of sustained directed motion, whereas at later times such directed motion became very infrequent. This sample age-dependence was consistent with the ensemble averaged dynamics of the particles, which showed a transition from large mean square displacements and non-Gaussian displacement distributions of beads at early times, to dynamics resembling that of passive (no myosin) samples at later times. The time-dependence of the active dynamics also matches the time-dependence of motor-driven coarsening of the actin network that I describe in *Chapter 3*. Detailed analysis of the translocation and relaxation of probe particles indicated that network coarsening is accompanied by large displacements of beads and plastic deformation of the network, as evidenced by incomplete relaxation. This study provides the first quantification of active network dynamics *in vitro* correlated with network structure, revealing the dynamic processes that lead to specific steady state configurations. It will be interesting to extend existing theories of active gel dynamics to account for the time-dependent and spatially inhomogeneous structure of actin-myosin gels and to explore implications of this coupling between structure and dynamics for the microrheology of cells.

In *chapter 5*, I focused on how pure physical constraints influence actin network organization. In addition to the influence of actin binding proteins, actin filaments are also subject to physical effects such as steric interactions imposed by the packing constraints of filaments at high actin density and the spatial confinement of filaments within the cell boundaries. I investigated how these physical constraints influence the spatial organization of dense solutions of actin filaments *in vitro*. Fluorescent actin was polymerized in cell-sized microchambers with nonadhesive walls at varying actin densities. Confocal micrographs revealed that actin filaments spontaneously organize into dense, bundle-like structures above a threshold concentration of 1 mg/ml. This contrasts with the behavior of unconfined networks, which are homogeneous and undergo a bulk isotropic-to-nematic phase transition above 5 mg/ml. In confinement, bundles align with the longest axis of anisometric chambers and with the diagonal in isometric chambers.

We propose a mechanism of bundling based on the concerted effect of quasi-2D confinement of the longest filaments in a mix of filaments with an exponential length distribution, and depletion interactions induced by the shortest filaments. The short filaments cause an attraction between the long filaments by a crowding (depletion) effect, and the long

filaments are confined in-plane. Quasi-2D confinement was sufficient to cause bundling in chambers with a depth comparable to the mean filament length. This mechanism differs from the isotropic-to-nematic transitions previously observed in bulk actin networks. We propose that the steady-state orientation of the bundles results from a competing liquid-crystalline ordering in the chamber center and alignment along the boundaries. This ordering effect of confinement might influence cytoskeletal organization in vivo. Cellular actin concentrations can be as high as 10 mg/mL which could promote local bundling in confining cell compartments such as the plant cell cortex or membrane protrusions.

I expect that building a more realistic actin-cytoskeleton could further help to elucidate the physical basis of cellular shape control. Combining confinement with polymerization of active actin-myosin gels would thus be a logical follow-up assay to understand the effect of boundaries mimicking the cell membrane on the active organization of the actin cytoskeleton. It will be important to modulate also network connectivity by adding crosslinking proteins or factors that regulate the filament length.